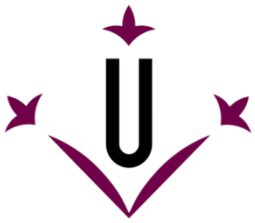

Assessment of soil fauna footprints at a rehabilitated coal mine using micromorphology and near infrared spectroscopy (NIRS)

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Abstract

Soil micromorphology in thin section and near infrared reflectance spectroscopy (NIRS) are useful techniques for assessing the participation of soil macrofauna in the formation of aggregates and soil structure. The purpose of this study was to use micromorphological analysis and NIRS techniques to assess the role of soil fauna in the recovery of soil aggregates and in the modification of soil microstructure in a chronosequence of rehabilitated areas at Cerrejon coal mine (La Guajira, Colombia). 64 soil samples were taken from rehabilitated areas (from 1 to 20 years ago) and from natural dry tropical forests Soil macroaggregates were subdivided into three categories: biogenic (BA), physical (PA), and non-aggregated soil (NAS). 32 samples were used for NIRS analysis, while ten thin sections of resin-impregnated soil blocks were micromorphologically analyzed. Principal component analysis of NIRS spectra showed a clear separation between BA, PA, and NAS. Likewise, an increase in BA was observed in the intermediate and advanced stages of rehabilitation. Respect to the micromorphological features, there was a clear change from a matrix of silt-sized quartz, unaccomodated peds, and unstructured materials with non-existent biogenic activity at the 2-year site to the formation of consolidated aggregates, more homogenized soil and increased biological activity at the 20-year site. Soil biological activity, principally the footprint of macrofauna, was recognized using the two techniques as well as at both the micro- and macro-morphology scales. These results reveal how the rehabilitation program being undertaken at Cerrejón mine is promoting the soil macrofauna population and associated bioturbation.

Key words: Soil invertebrates, soil thin sections, soil aggregates, bioturbation, Colombia, Technosol, pedogenesis, mine reclamation.

Introduction

Open-pit coal mining involves completely removing the vegetation and soil from an area for mineral extraction, impacting strongly on soil, water, and biodiversity. For this reason, rehabilitation programs must ensure the restoration of environmental goods and services in affected areas (Cooke and Johnson, 2002). Ecological rehabilitation in these mines, is carried out on spoil heaps covered by natural topsoil coming from recently dug areas (Sheoran et al., 2010; Macdonald et al., 2015). This type of soil is denominated a Technosol (Rossiter, 2007; IUSS Working Group WRB, 2015), in the case of Cerrejon mine in Colombia, more that 60% of the upper 100 cm from the soil surface are constituted by spoil mine. These are disposed for allow revegetation

Soil organisms play a fundamental role in pedogenetic processes in soils (Brussaard, 1998; Jiménez and Decaëns, 2004; Jiménez and Lal, 2006; Lavelle et al., 2006, 2016; Frouz et al., 2008). In particular, soil macrofauna, e.g., invertebrates >2 mm in body size (Swift et al., 1979) participate in the stabilization and degradation of aggregates (Jones et al., 1994; Lavelle, 1997; Decaëns, 2000; Zangerlé et al., 2011; Bottinelli et al., 2015), biopore formation (Colloff et al., 2010; Bottinelli et al., 2015), and play an important role in soil organic matter (SOM) dynamics (Filser et al., 2016). Soil aggregates, as produced by some earthworms, can be considered trophic resources for other organisms l as they are C reservoirs (Jiménez and Decaëns, 2004), and the largest may affect soil processes such as erosion (Jouquet et al., 2010) and water infiltration (Colloff et al., 2010)

The role of soil macrofauna in the formation of aggregates and soil structure can be demonstrated by two complementary techniques: soil micromorphology and near infrared reflectance spectroscopy (NIRS). Micromorphology is a basic tool widely used to diagnose soil-

forming processes (Stoops, 2003).It is a useful technique for understanding the role of macrofauna in biogenic processes related to the decomposition and redistribution of organic matter (OM), humus development and formation, pore formation, and the link with inorganic soil components (Kubiëna, 1964). Voids, excrements, coatings and infillings, are the mains micromorphological features resulting from soil fauna, that may be analyzed by thin sections (Kooistra and Pulleman, 2010), It has been used to study changes in soil function in mining areas, through a soil rehabilitation chronosequence (Arocena et al., 2010; Frouz et al., 2013) and other soils under different land use (Pulleman et al., 2005).

Near-infrared reflectance spectroscopy (NIRS) is a rapid and non-destructive analytical technique for characterizing the molecular composition of OM, involving diffuse-reflectance measurements in the near infrared region (750 – 2,500 nm) (Joffre et al., 2001). NIRS is a high-precision, and low-cost method applied in soil science (Ben-Dor and Banin, 1995; Bellon-Maurel and McBratney, 2011). This method has been used to: 1) discriminate between different types of aggregates (biological, physical and root) using multivariate ordination methods (Velasquez et al., 2007); 2) identify the organisms that produce the aggregates (Decaëns et al., 2001; Hedde et al., 2005; Cécillon et al., 2008; Zhang et al., 2009; Jouquet et al., 2010; Zangerlé et al., 2011; Huerta et al., 2013) based on the specific NIRS signature of each biogenic soil structure, different from that of the surrounding soil; and 3) study soil quality (Velasquez et al., 2005; Cécillon et al., 2009).

Technosols in coal mines start as disintegrated and mixed edaphic materials. Given the significant role of soil biota in the pedogenetic processes related with horizon formation (Arnold and Williams, 2016; Leguédois et al., 2016), it is necessary to evaluate not only the recovery of

soil macrofauna diversity per se, but the reestablishment of their functions, to understand how these organisms contributed to the restoration (Bottinelli et al., 2015).

This study used micromorphological analysis and NIRS techniques to evaluate the role of macrofauna in the recovery of the physical structure of soil in a chronosequence of rehabilitated areas in a Colombian coal mine. It was hypothesized that the formation of biogenic aggregates, pores, and pedofeatures created by the activity of soil macrofauna will be associated to intermediate and advanced stage of rehabilitation. The micromorphological results are based on qualitative observations. Thus, the aims of this study were: (i) to evaluate the sensitivity of the NIRS technique for recognizing aggregate types obtained from areas with different rehabilitation ages; and (ii) to analyze macrofauna contribution in several soil development processes through macro and micromorphological observations of soil thin sections.

1. Materials and methods

2.1 Study area

Cerrejón coal mine is situated in the La Guajira Department, northern Colombia (11°3'N, 72°44'W - 11°8'N, 72°37'W) at 200 to 240 masl. The natural surrounding ecosystem is seasonal dry tropical forest with sub-xerophytic vegetation dominated by *Prosopis juliflora* (Sw.), *Bulnesia arborea* (Jacq) Engl., *Platymiscium pinnatum* (Jacq) Dugand, *Cereus repandus* (L.), *Tabebuia billbergii* (Bureau & K. Schum.), *Bourreria exsucca* (L). Jacq., *Astronium graveolens* Jacq., *Guazuma ulmifolia* Lam. and the liana *Serjania* sp. The soil is classified as an Entisol. The climate is warm and dry, with a yearly average temperature of 27.5^{o}C (isohyperthermic). The precipitation is bimodal (March-April and October-November) with an annual mean of 800 mm, and evapotranspiration from 1000 to 1500 mm.

The rehabilitation processes in the area can be summarized in the following stages: (i) topographical reconstruction of spoil heaps; (ii) spreading and covering the mine spoil with approximately 30 cm of transported soil from natural areas (recently affected by mine activity), specifically from Forest 2; (iii) soil stabilization with buffel grass (*Cenchrus ciliaris* L.); and (iv) revegetation with diverse native plants, principally from the Leguminosae family (Domínguez-Haydar and Armbrecht, 2011; Gualdrón, 2011).

Seven areas, rehabilitated for 1, 2, 6, 8, 9, 16 and 20 years, respectively, were selected following a chronosequence (space-for-time approach), and two forested areas unaffected by mining were selected as reference areas (Forest 1 and Forest 2). This design is common in studies conducted in rehabilitated mine areas (Arocena et al., 2010; Domínguez-Haydar and Armbrecht, 2011; Kuráž et al., 2012; Mukhopadhyay et al., 2014). In each area, sample soil points were located outside of permanent monitoring plots (10 x 25 m), which are selected by the mine according to the types of vegetal coverage. We selected the plots with more representative characteristics of areas. The table 1 summarized the analyses performed in each area, while soil physical and chemical properties from the selected areas are listed in Table 2.

2.2 Soil macrofauna

Data of soil macrofauna were taken for to explain the results fo NIRS and micromorphologycal analyses. From sites of 1-, 6-, 9-, 20-y and Forest, two soil monoliths of 25 cm x 25 cm x 20 cm in depth were taken from each monitoring plots (4 plots) and handsorted for macroinvertebrates (ISO 23611-5:2011). Identifications were done at the order or class (Oligochaeta, Chilopoda and Diplodopa) and level according to the identification keys of Borror et. al (1976). Only a general description of richness and density were performed.

2.3 Chemical analyses

Soil chemical variables were assessed using standard methodologies: Total phosphorous (P) was measured by UV-VIS L-ascorbic spectrophotometry, Ca, Mg and K by atomic absorption (Ammonium acetate 1 N), pH (in a 1:2 v/v water solution), organic matter (OM) content by Walkley and Black volumetric method (Walkley and Black, 1934), total nitrogen by a Micro-Kjeldahl method and effective cation exchange capacity (ECEC) was estimated as the sum of cations extracted with Ammonium acetate 1 N (Burt, 2004).

2.3 Soil macroaggregates and NIRS

At each site (Table 1), four permanent monitoring plots (10 x 25 m) separated by a minimum distance of approximately 300 m were selected. Outside of each plot, two soil block samples (10 x 10 x 10 cm), placed on opposite corners were taken; the macroaggregates (>5 mm) were manually separated, air-dried, weighed and classified following the protocol described in Velasquez et al. (2007): (i) biogenic aggregates (BA), recognized as rounded forms, pores and concave structures; (ii) physical aggregates (PA), characterized by angular shapes and a smoother structure; and (iii) non-aggregated soil (NAS). Additionally, root aggregates (RA), organic debris (OD) and stones (St) were also analyzed (hereafter BA, NAS and PA will be referred to collectively as macroaggregates; the nomenclature provided by is not followed here when the word macroaggregate is used explicitly).

For NIRS analysis, the macroaggregate samples (BA, PA) and NAS were taken from one of two block used for anterior analysis (a total of four blocks per site). From each block, two biogenic and/or physical aggregates (3 g), were randomly taken. In the case of the NAS, two 5 g samples were taken. A total of 16 (BA and PA were not present simultaneously) samples for site were

separated for this analysis. The samples were air dried, macerated, passed through a 500 μm sieve, and placed in a quartz glass container (Zangerlé et al., 2014). The NIRS signatures were read in a spectrophotometer (NIRFLEX N 500, Buchi®) with a 700 to 4000 nm spectral range. NIRS spectra values were obtained in the near infrared region between 1040 and 2510 nm at 8 nm intervals. Each value of the NIR spectra was then transformed with second derivative procedure as spectral preprocessing (Savitzky and Golay, 1964) using the Unscrambler X 10.3 software, according to techniques recommended to remove baseline shifts and reduce noise (Jouquet et al., 2014; Zangerlé et al., 2016).

2.4 Topsoil micromorphology

In this study, four sites were selected (6-, 9-, 20-y and Forest 1) and a site of 2-y to replace the 1-y site, as the soil was not consolidated in the latter. These sites corresponded to initial, intermediate and advanced rehabilitation stages (Table 1). Undisturbed soil samples were collected from the topsoil layer (0 to 8 cm) using 8 x 8 cm Kubiena boxes for micromorphological analysis. Two replicated soil samples were taken from each of the selected sites. The soil blocks were dried in an oven at 40 ºC for 7 days and then impregnated with a cold-setting polyester resin. Thin sections were prepared using the method of Guilloré and described following Stoops (2003), these were analyzed and photographed under plain polarized light (PPL) with a petrographic microscope (Nikon eclipse LV100POL). Additionally, the soil sections were illuminated under white light and photographed using a digital camera (Canon EF50 mm Macro lens). Macroscopic description of the thin sections was undertaken with the naked eye.

2.4 Data analysis

A principal component analysis (PCA) was performed on the data matrix of the soil macroaggregate samples (BA, PA and NAS) containing 6 columns (BA, PA, NSA, OD, St and RA) and 64 rows (sites), followed by a discriminant analysis and a randomization test (Monte Carlo procedure, 999 permutations) to search for significant differences among sites (Manly, 1991).

Later, two PCA were carried out on the matrices with the NIR spectra values, one according to aggregate type (biogenic, physical and non-aggregated), independently of sampling site, and another one including aggregate type and site. The main sources of variation and significance were investigated with a between-class PCA (Chessel et al., 2004), a specific multivariate ordination technique that focuses on between-groups' differences, i.e. aggregate type (BA, NA, PA) across sites. As in a normed PCA this between-class PCA gives a score that maximizes the sum of squared correlations with variables. The corresponding between-class PCA analysis is done with the between function (bca), in which a one single factor defines the groups, i.e. the sites. Therefore, this is a between-sites analysis, aiming at discriminating the restored sites and forests, given the distribution of aggregates' types. For further details on the use and interpretation of this between-class PCA see (Dolédec and Chessel, 1989). Again, the significance of the ordination was tested with a Monte Carlo randomisation procedure with 999 simulations (Manly, 1991). These analyses were carried out using the ADE 4 package (Thioulouse et al., 1997) in R software, version 3.0.1 (R Development Core Team, 2014).

2. Results

3.1 Soil macrofauna

Forest 2 showed the highest taxa richness and density (18 groups, 270 ind.m^{-2} Table 4), followed by 20- (18 groups, 176 ind.m^{-2}), the 1-y site showed the least richness and density (7 groups, 20 ind.m^{-2}) Coleoptera, Hymenoptera, Isopoda and Isoptera were the groups with the highest densities.

3.2 Chemical analyses

Variation in chemical values through cronosequence was low, significant differences were observed among forest and 1-yr, the forests showed the highest values of OM, and N, and the lowest in pH (Table 2). However, among the forests and areas with intermediate and advanced areas neither differences were found.

3.3 Visual separation of macroaggregates

Biogenic aggregates increased with rehabilitation time. Statistically significant differences ($P < 0.001$) were observed among forests and more advanced rehabilitated areas with respect to 1-y site. These showed an absence of PA and high values of BA and OD. (Table 2).

In the PCA of macroaggregate types (Fig. 1), the first two components contributed with 54% of the total variability. Axis 1 (32% of the explained variance) significantly separated the 1-y site, characterized by high physical aggregate values compared with the other areas that had higher biogenic aggregate values (Fig. 1). The second axis (22% of total variance explained) distinguished the areas according to organic debris, NAS and stone contribution. The forests were characterized by a greater content of organic debris and root aggregates, while an abundance of stones was observed at the 16-y site. The Montecarlo permutation test showed that site separation based on macroaggregates was significant ($p < 0.01$), with 35% of the total variance explained by this factor.

3.4 NIRS analysis of biogenic structures

The ordination based on magroaggregate type was significant: the between-class PCA clearly separated BA, PA and NAS (Montecarlo test, 12% of total variance explained, $p < 0.01$) in spite of the fact that there was a high degree of dispersion between the groups (Fig. 2A). The first axis explained 68% of the total variance and separated BA from NAS, while the second axis (31% of the total variance), separated the PA from the other two macroaggregate types.

The between-class PCA for aggregate type and sites (Fig.2B) was significant, making a higher contribution to total variance (39%). The first factor (29% of total variance) separated the groups by site, while the second factor (24% of total variance explained) separated the groups according to aggregate type. Nevertheless, the biogenic aggregates of the 8-y and 9-y sites were grouped together with NAS, the physical aggregates were discriminated from the group of BA and located closer to the NAS group.

3.5 Main micromorphological features

Soil from the 2-y site comprised very coarse soil aggregates (> 1 cm). The most active soil process seemed to be loss of structure and subsequent eluviation of materials (Fig. 3a). Silt and sand-sized particles had been translocated through the upper soil layer, together with microaggregates inherited from the "original soil". The translocated material formed loose infillings in the voids and frequent cappings, i.e., coatings on top of grains and aggregates (Fig. 4a). These cappings consisted of loose and unstructured materials detached by mechanical action. Illuviation due to rain caused particles to move down from the soil surface. In agreement with the characteristics of the exchangeable cations (Table 2), there was no evidence of soil dispersion produced by chemical processes. Coarse-grained coatings have been reported in other environments such as frost-affected soils and have also been related to the rapid wetting of dry

soils (Kühn et al., 2010). The soil contains relatively fresh roots which exhibit a clear blue primary fluorescence in lignin parts. They have not lost the structure and there are no tissues containing phlobaphene, that is, products of oxidation and polymerization of tannins, which are conspicuous by a high chroma (Bullock et al., 1985). Roots are not ravaged by oribatids, and there is a clear geometric parallelism between the root and the walls enclosing it (Fig. 4b). Chambers or channels from current biogenic activity were not found (Table 5).

The next soil stage, at the 6-y site, was noticeably laminar with a platy microstructure and planar voids. Similar to those of the 2-y site, the soil aggregates were coarse (centimeter to decimeter in size) and included roots with tissue remains and channels 8-10 mm in diameter. All these biogenic features were features of the aggregates from the "original soil" (Fig. 3b). There was increased development of loose infillings and cappings (Fig. 4b, c), some of which exhibited internal organization involving grain size grading indicating different episodes of growth (Table3).

The soil aggregates at the 9-y site indicated a degree of reorganization within the soil structure. Together with some planar voids, a new porosity had developed due to a marked increase of the in situ biological activity. This was evidenced by the occurrence of frequent isolated ellipsoidal and coalescent earthworm fecal pellets, up to 3 mm in diameter, and similar-sized channels (Fig. 3c). There is also new system of living roots (Fig 5B) and soil biological activity distorting the planar structure". This biological activity had promoted the reorganization of the soil into small subangular blocky peds (Fig. 5a), very reduced in size compared with those seen in previous soil stages (2-y and 6-y sites). The loose infilling of voids was very common, sometimes displaying crescent or bow-like forms indicating episodic accretion (Fig. 5c, d). The planar voids showed

partial infilling with microaggregates and cappings were still frequent, together with channels and chambers (Table 5).

The 20-y site presented a more advanced stage of soil homogenization than the previous 9-y soil. The aggregates were well defined and more integrated with the sandy material, and a laminar microaggregation was observed in some areas. The blocky peds that were subangular in the previous stages were more rounded at this site and the soil structure tended to be granular with smaller voids. There were infrequent remnants of the platy microstructure from the 6-y site, and the occurrence of few isolated relict cappings indicated that the processes related to loss of structure in the soil were much less active (Fig. 6a, Table 5)

Loose channel infills, up to 2 mm in diameter, were observed generated by small earthworms, as well as mammilated microaggregates (Bullock et al., 1985) enriched in organic matter, also indicating faunal activity, and tuberous mineral excrements (Fig. 6b, c, d). The local occurrence of self-mulching at the soil surface, probably favored by expansive clays, also evidenced a clear stage of soil reorganization (Table 5).

The soil from the forest sites consisted of small peds similar to those found in the 20-y soil, and varied from a subangular blocky to granular complex structure (Figure 7, Table 5). The biological activity was much more important in these locations, with frequent pellets produced by insects and oribatids adjacent to vegetal remains, mainly root tissues. The frequent occurrence of charcoal fragments (some preserving the tissue structure) indicated anthropic activity (Table 5).

3. Discussion

The macroaggregate analysis revealed a rapid recovery of soil physical morphology through biological activity evidenced by the change from PA at the 1-y site to BA in the rest of the chronosequence (Fig. 1). The nature of these aggregates was corroborated using NIRS analysis, which allowed that BA to be distinguished from NAS and PA, thse results confirmed the application of technique of manual separation of aggregates (Velasquez et al., 2007; Zangerlé et al., 2016) under mine scenarios. This makes it possible to differentiate biological processes from physical ones, the BA production were related with the presence of macrofauna, which increased its richness and density in intermediate and advances stages of rehabilitation (Table 4). This is in agreement with other studies, where PA have been associated with annual crops or degraded pastures, which are poor in soil fauna (Velasquez et al., 2007; Lavelle et al., 2014).

Despite of the high degree of variability characterizing Technosols, the first PCA component, performed on the NIRS wavelengths discriminated the aggregates according to their origin (Fig 2b), it was possible to discriminate aggregates of unknown formation time and origin (i.e., which organism formed them), given that in six out of eight sites, BA was clearly separated. This technique reliably identified fresh aggregates produced by earthworms (Jouquet et al., 2009) and other structures generated by ecosystem engineers (Hedde et al., 2005). However, the 8-y and 9-y sites were jointly located to the NAS group in the PCA factorial plane, under field conditions, the usefulness of NIRS may depend on local characteristics, such as the amount of OM, macro-porosity and heterogeneity (Bottinelli et al., 2013; Zangerlé et al., 2016), however, it is not possible to explain why this occurs only at these sites.

The second component (Fig 2b), separated the forests from rehabilitated areas, this separation was due to the highest content of OM. In consequence, NIRS could be useful for determining soil quality in affected areas (Velasquez et al., 2005; Cécillon et al., 2009). Changes in both the

quantity and quality of SOM can be recognized through spectral signatures (Velasquez et al., 2005; Zangerlé et al., 2014).

In the Technosols of the Cerrejon coal mine, there was a clear evolution from unaccomodated peds, planar and simple voids, frequent loose infilling, and translocation of soil particles, observed in the initial stages to the formation of consolidated aggregates and better pore system at the 20-y site (Table 5). The changes observed in the microstructure and the textural pedofeatures are related to biotic activity. The absent of current excremental pedofeatures and other signs of biotic activity in the 2-y sample, followed by the presence of dark microaggregates (excrements) into the faunal passages (Fig. 3c) at intermediate and advanced stages of rehabilitation, are an example of bioturbation processes.

This soil evolution coincides with others natural revegetation scenarios. At the beginning there are mineral spoils, later leaf litter accumulates and is transformed by microbial processes and soil mesofauna, followed by the accumulation of macrofaunal fecal pellets as was observed by Frouz and coworkers (2007b). There was a reorganized structure in terms of porosity (void complex) and aggregation (granular complex) at the 20-y site. In “Terra Preta” Anthrosols (Cunha et al., 2016) much of the granular microstructure and simple packing voids are due to faunal bioturbation.

Micromorphology studies have led to the recognition of the role soil fauna plays in soil development in rehabilitation areas (Frouz et al., 2006; Davidson et al., 2007). As expected, the footprint of soil fauna, recognized as excrement, infilled galleries and vughs (Kooistra and Pulleman, 2010), was found in those areas in intermediate and advanced stages of rehabilitation. Macrofaunal activity together with root development contribute to more complex voids and a

well-developed granular microstructure, which indicate good quality, productive soil (Arocena et al., 2010).

Another important aspect from the micromorphological observations is the incorporation of OM into the soil. Humification processes were evident from the 6-y sample on. This condition, together with the action of soil fauna, results in SOM accumulation. The translocation of organic matter to the mineral layer as a result of faunal mixing activity in the soil was also apparent in infilling galleries (Frouz et al., 2007; Frouz, 2014). This corresponds with the slight increase in the OM observed along the chronosequence (Table 2).

In addition to plant recovery through rehabilitation action (Domínguez-Haydar and Armbrecht, 2011), belowground processes driven by macrofauna, related to bioturbation and soil aggregation, were evidenced by the NIRS and micromorphology analyses. Several functions may be inferred from this, such as improvement in the soil's physical properties by bioturbation (Wilkinson et al. 2009), water holding, aeration capacity (Lavelle, 1997), mineralization of OM, and the accumulation of C in the soil (Frouz et al., 2007). However, this is an initial approach because our results are limited to the first 10 centimeters of the upper soil layer. To understand the physical, chemical and biological process that participate in the reorganization of the soil profile, a more detailed study of the subsurface soil horizons is required (Davidson et al., 2007).

4. Conclusions

The usefulness of NIRS for differentiating biogenic structures was demonstrated in mine Technosols. These aggregates were associated with intermediate and advanced rehabilitation stages and forest areas, which has implications for monitoring programs. The soil fauna contributed to the formation of complex pores, a granular structure and stable soil aggregation. The accumulation of SOM in areas of advanced rehabilitation and the forest sites, in addition to

the predominance of non-biogenic aggregates associated with the initial stages of soil development were observed using both micromorphology and NIRS techniques.

Although NIRS has been recommended over soil micromorphological analysis (Zangerlé et al., 2016), each has its own peculiarities meaning they are not direct substitutes for each other. The micro-morphological approach enables both physical and chemical pedogenetics processes to be recognized, but in a reduced number of samples, while NIRS can detect chemical signatures in a greater quantity of samples. Both approaches are important for revealing macrofaunal activity, even if the fauna themselves are absent from the samples, i.e., during the dry season.

The footprint of soil fauna in the soil development was recognized using the two different techniques and at both scales (micro- and macromorphology), these results show how the rehabilitation action being carried out at Cerrejón mine is promoting the return of soil organisms and the restoration of ecosystem services related to nutrient cycling and bioturbation.

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Tables

Table 1. Site description and indication of studies performed in each site.

Site	Habitat description and dominant species	Canopy cover (%)	Study performed[¥]
1-y	Bare soil, dispersed patches of spontaneus grasses. Whitout revegetation actions	0	MS, NIR
2-y	Revegetation with *Cenchrus ciliaris* L. (Buffell grass), mixed with other spontaneus grasses.	0	TS
6-y	Intermediate densities of grass *C. ciliaris* and scattered trees (3 m tall) of *Mimosa arenosa, Acacia macracantha* Will. and *Acacia tortuosa* (L.) Will.	52.8	MS, NIR, TS
8-y	High *C. ciliaris* and tree densities with large canopies (4 – 5 m). *M. arenosa, A. macracantha*, *A. tortuosa* , *Caesalpinia ébano* H.Karst.	55.5	MS, NIR
9-y	High *C. ciliaris* and tree densities with large canopies (4 – 5 m) *M. arenosa, A. macracantha*, *A. tortuosa* , *Caesalpinia ébano* H.Karst.	75.3	MS, NIR, TS
16-y	Major canopy cover, diminished density of grasses. *M. arenosa, A. macracantha*, and *Caesalpinia ébano* H.Karst. *Prosopis juliflora* (Sw.),	94.1	MS, NIR
20-y	Mosaic of bare soil, some scattered patches of grasses and litter. *M. arenosa A. macracantha*, *Caesalpinia ébano, P. juliflora*, *Platymiscium pinnatum* (Jacq) Dugand,	93.1	MS, NIR, TS
Forest 1	Sub-xerophytic vegetation dominated by *P. juliflora*, *Bulnesia arborea* (Jacq) Engl., *Platymiscium*, *Cereus repandus* (L.), *Tabebuia billbergii* (Bureau & K. Schum.), *Bourreria exsucca* (L). Jacq., *Astronium graveolens* Jacq., *Guazuma ulmifolia* Lam. and the liana *Serjania* sp.	92.2	MS, NIR, TS
Forest 2	*Aspidosperma polyneuron* Müll.Arg. and *Hura crepitans* L. The shrub layer: *Cordia alba* (Jacq.) Roem. & Schult., *Machaerium* sp. and *Mimosa arenosa*; some grasses product of cattle activity, 40 years ago.	99.6	MS, NIR

[¥] MS= Macroaggregate study, NIR= near infrarred reflectance spectroscopy, TS= Thin section technique (micromorphology analysis).

Table 2. Mean values and standard error (italic) of chemical properties in rehabilitated areas and in the undisturbed forests. Kruskall Wallis test was performed and a posthoc comparisons using Dunn test was done.

Variables/ Age (y)	**1**	**6**	**8**	**9**	**16**	**20**	**Forest-1**	**Forest-2**
pH	8.18	8.15	8.08	8.17	8.17	8.13	7.78	7.46
	0.04	*0.08*	*0.06*	*0.05*	*0.04*	*0.06*	*0.15*	*0.22*
Ca (meq)	57.62	49.46	45.12	46.98	53.04	44.83	45.98	40.99
	14.7	*15.9*	*15.4*	*15.04*	*16.1*	*17.52*	*19.1*	*16.2*
Mg (meq)	6.20	5.33	5.28	5.21	4.85	5.34	3.94	4.75
	0.47	*0.61*	*0.61*	*0.55*	*0.43*	*0.85*	*0.30*	*0.49*
K (meq)	0.94	1.29	1.09	1.51	1.44	1.30	1.12	1.61
	0.13	*0.21*	*0.21*	*0.32*	*0.31*	*0.28*	*0.30*	*0.52*
CECe	64.77	56.09	51.50	53.71	59.34	51.48	51.05	47.34
	16.34	*17.74*	*17.83*	*16.84*	*17.69*	*18.75*	*20.85*	*18.16*
OM (%)	2.24	2.71	2.58	2.77	3.06	3.05	4.08	3.93
	0.23	*0.22*	*0.25*	*0.68*	*0.30*	*0.28*	*0.31*	*0.28*
N (%)	0.11	0.13	0.13	0.14	0.16	0.15	0.21	0.20
	0.01	*0.01*	*0.01*	*0.03*	*0.01*	*0.02*	*0.01*	*0.01*
BD (g cm^{-3})	1.32	1.2	1.31	1.2	1.23	1.21	1.08	1.15
	0.11	*0.14*	*0.12*	*0.06*	*0.12*	*0.05*	*0.11*	*0.08*
Sand (%)	38.75	41.25	45.25	44.25	42.75	41.75	43.5	43
	6.41	*9.13*	*5.55*	*5.99*	*4.89*	*4.33*	*12.08*	*12.04*
Silt (%)	34	31	30	32	33	33	32	35
	8	*8*	*5*	*6*	*7*	*3*	*7*	*10.68*
Clay (%)	28	28	25	24	24	26	24	22
	5	*5*	*6*	*3*	*3*	*3*	*8*	*3.02*

Table 3. Mean values and standard error (italic) of morphology properties in rehabilitated areas and in the undisturbed forests. Kruskall Wallis test was performed for macroaggregates and other variables, and a posthoc comparisons using Dunn test was done.

Variables/								
Age (y)	1-y	6-y	8-y	9-y	16-y	20-y	Forest 1	Forest 2
NAS (g)	369.19 a	538.85 ab	569.65 ab	536.04 ab	629.46 b	429.16 ab	521.29 ab	445.55 ab
	75.66	*177.4*	*115.53*	*73.58*	*106.31*	*119.01*	*271.98*	*74.27*
BA	0 a	393.7 b	579.57 bc	614.43 bc	608.26 bc	735.22 c	669.41 c	545.27 bc
	0	*192.34*	*118.87*	*190.01*	*199.45*	*194.02*	*191.39*	*112.1*
PA	903.87 a	48.93 a	7.02 bc	1.64 bc	9.59 bc	12.77 bc	0 c	0 c
	150.67	*99.65*	*10.77*	*4.63*	*10*	*24.22*	*0*	*0*
OM	1.43 a	1.83 ab	2.09 ab	2.88 ab	2.39 ab	1.6 ab	3.07 b	7.42 b
	1.71	*0.86*	*1.13*	*2.2*	*1.81*	*0.86*	*2.3*	*3.01*
Stones	14.39	29.7	18.17	18.77	66.3	33.26	10.33	4.11
	10.45	*30.03*	*21.93*	*27.99*	*82.66*	*52.73*	*18.47*	*6.21*

Table 4. Mean values and standard error (italic) of macrofauna in some rehabilitated areas and in the undisturbed forests.

	1-yr	6-yr	9-yr	20-yr	Forest
Aranae	0	4	4	6	22
	0	*1.85*	*1.85*	*2.98*	*7.07*
Chilopoda	4	2	0	6	2
	1.85	*1.41*	*0*	*2.98*	*1.41*
Coleoptera	4	8	16	40	30
	1.85	*3.02*	*6.41*	*6.05*	*9.43*
Coleoptera-L	4	2	56	6	22
	2.83	*1.41*	*31.06*	*2.98*	*6.39*
Diplopoda	0	0	6	16	8
	0	*0*	*2.98*	*5.24*	*3.02*
Embioptera	0	0	0	2	28
	0	*0*	*0*	*1.41*	*9.74*
Hymenoptera	2	2	10	20	36
	1.41	*1.41*	*4.75*	*9.5*	*14.46*
Isoptera	2	2	4	44	18
	1.41	*1.41*	*2.83*	*12.78*	*5.42*
Isopoda	0	18	2	4	56
	0	*6.21*	*1.41*	*1.85*	*31.71*
Lepidoptera	4	8	12	6	8
	1.85	*3.02*	*4.66*	*2.98*	*3.02*
Oligochaeta	0	4	0	6	6
	0	*2.83*	*0*	*4.24*	*2.07*
Thysanura	0	14	6	18	14
	0	*6.91*	*2.98*	*8.12*	*3.96*
Others	0	0	4	2	20
	0	*0*	*1.85*	*1.41*	*5.55*
Density	**20**	**64**	**120**	**176**	**270**
Richness[¥]	**7**	**11**	**13**	**16**	**18**

[¥] Number of taxa or taxonomical groups

Table 5. Soil micromorphology description for technosols of "Cerrejon" coal mine

		GROUNDMASS						Pedofeatures		
Sample # (soil age)	**MICROSTRUCTURE**	**POROSITY**	**coarse/fine limit and percentage**	**Related distributio n pattern**	**Coarse material mineral**	**Coarse material organic**	**MICROMASS**	**Textural**	**Excremental**	**Cristal**
(2 years)	Subangular blocky with cm-sized peds (Clods, see Bullock)	a) the intra aggregate porosity shows a trend to planar voids b) extra aggregate porosity: packing voids, simple for sand grains and compound for microaggregates	3 mm, c/f = 50/50	Single space enaulic and partially porphyric	- subrounded quartz grains (m.s.), dominant -grains of carbonates (f.s.), few - aggregates		crystallitic b-fabric, Occasionally, the aggregates show a certain birefringence with a crystallitic speckled b-fabric	Infillings with capping	Mineral: Loose continuous infillings Organic: pellets from oribatids (inherited from original soil)	Typic carbonate nodules
(6 years)	Apedal groundmass and partially subangular blocky	a) predominant planar voids and channels b) Compound packing voids (Idem 2A)	2 mm, c/f = 80/20	Porphyric	- subrounded quartz grains (m.s.), dominant - aggregates		crystallitic b-fabric	Infillings with capping	Loose continuous infillings Organic: pellets from oribatids (inherited from original soil)	Typic carbonate nodules
(9 years)	Subangular blocky with predominant mm-sized peds and few cm-sized peds.	Conspicuous planar voids, and channels	10 µm, c/f = 90/10	Porphyric	- subrounded quartz grains - aggregates		crystallitic b-fabric Yellowish brown	- Infillings with capping	Loose continuous infillings Organic: pellets from oribatids	Typic carbonate nodules Carbonate pseudomor phes
(20 years)	Granular and complex Locally subangular blocky	Compound packing voids and planar voids	10 µm, c/f = 90/10	Porphyric	- silt coarse - subrounded quartz grains - fragments of ferruginized claystones	2-3% Roots remains	crystallitic b-fabric	Infillings with capping	Loose continuous infillings Organic: pellets from earthworms, oribatids and insects	Typic carbonate nodules Carbonate pseudomor phes
Natural forest	Granular and complex Locally subangular blocky Small peds	Compound packing voids and planar voids	10 µm, c/f = 90/10	Porphyric	-subrounded quartz grains (f.s.-m.s.),frequent; -subangular carbonate fragments (m.s -gravel), ferruginized - fragments of ferruginized claystones	< 1% charcoal	crystallitic b-fabric	Infillings with less amount of capping	Loose continuous infillings Organic: pellets from earthworms, oribatids and insects	- Typic carbonate nodules

Figure captions

Fig. 1. A) PCA biplot of macroaggregates variables and projection of study areas in the plane defined by the first two factors. B) Same ordination including those significant chemical variables, Nitrogen content, organic matter and pH. NSA=Non soil aggregate, BA=Biogenic aggregate, PA=Physical aggregate, RA=Root aggregate, OD= Organic debris, St= Stones.

Fig. 2. A) Projection of NIRS spectra of macroaggregates type from forests and rehabilitated areas in the plane defined by the first two factors of PCA. B) Projection of barycenters of NIRS spectra grouped by site and type of macroaggregates in the plane defined by the first two factors of PCA – between. BA = Biogenic aggregates, NAS = non-agreggrate soil and PA = Physical aggregates. 1, 5, 8, 9, 16, 20 = Sites with different age of rehabilitation. F = Forest.

Fig. 3. Photographs of whole thin section (White light. Canon EF50 mm Macro lens). A) 2-y site, B) 6-y site, C) 9-y site and D) 20-y site.

Fig. 4. A) Loose infilling with sand-sized particles and microaggregates (2y soil, plain polarized light = PPL); B) Root remain with associated oribatid pellets, within an aggregate inherited from the "previous soil" (2y soil, PPL); C) Layered (grain selection) capping (6y soil, PPL); D) Capping examples (6y soil, PPL).

Fig. 5. Micromorphological features of 9y soil (PPL): A) Microstructure re-organized: from platy to subangular blocky; B) Remains of new alive roots; C) Infilling and bow-like (crescent shape) channel; D) Loose infilling of fauna dejection (pellets) in a channel.

Fig. 6. Micromorphological features of 20y soil (PPL): A) Persistent capping on the top of a disaggregated (broken) ped; B) Faecal aggregates over 1-2 mm size with a tuberous morphology; C) Root remains and granular microstructure, locally subangular blocky; D) Loose channel infilling with microaggregates.

Fig. 7. Selected micromorphological features of forest soil resulting from soil fauna activity (PPL). A) Bow-like loose infilling of a centimeter-long faunal channel; B) Detail of the loose infilling showing a sharp external boundary and the subangular blocky microstructure around the channel; C) Root remain with faecal pellets from oribatids showing some degree of coalescence; D) Prismatic faecal pellets from insects.

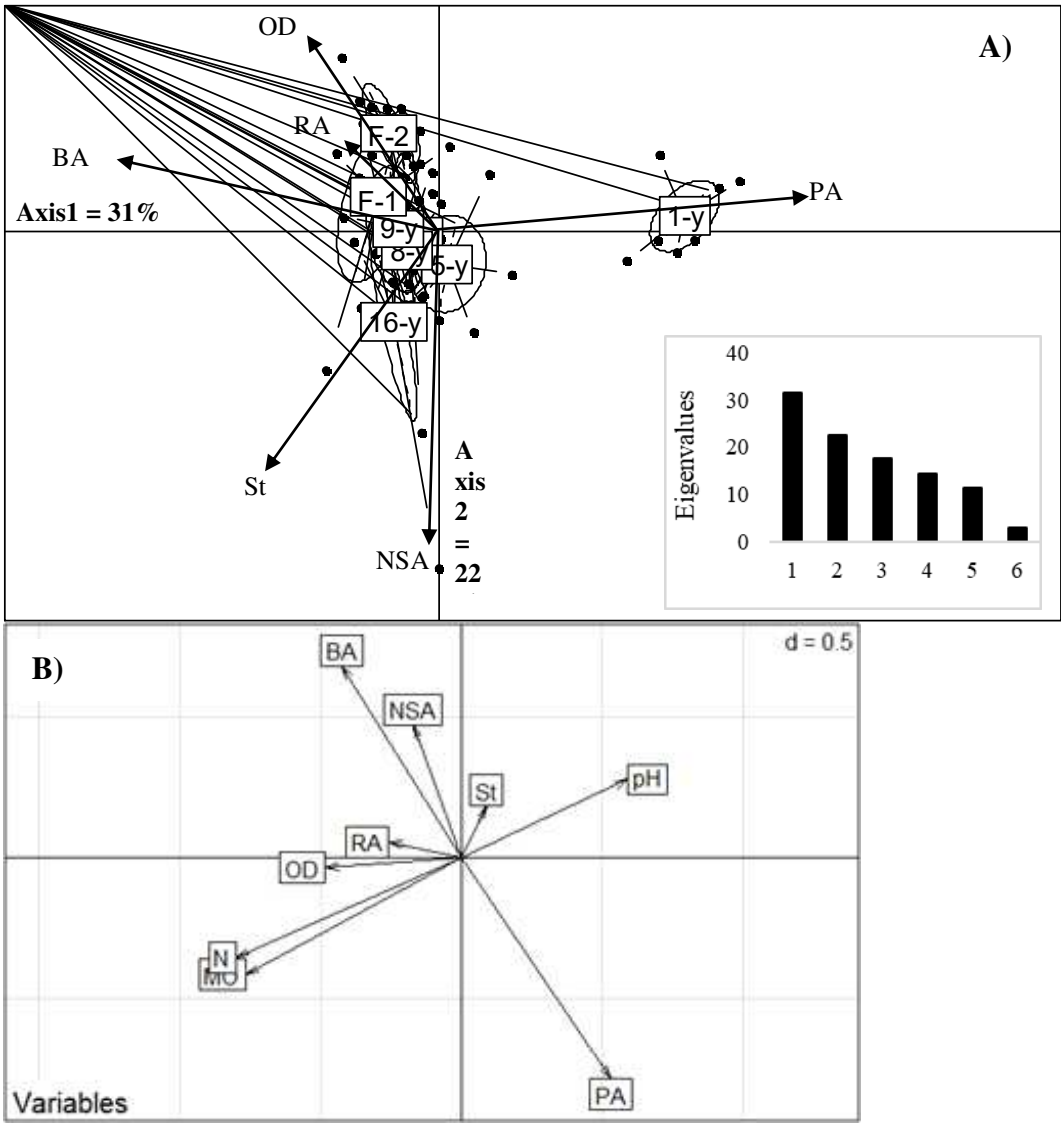
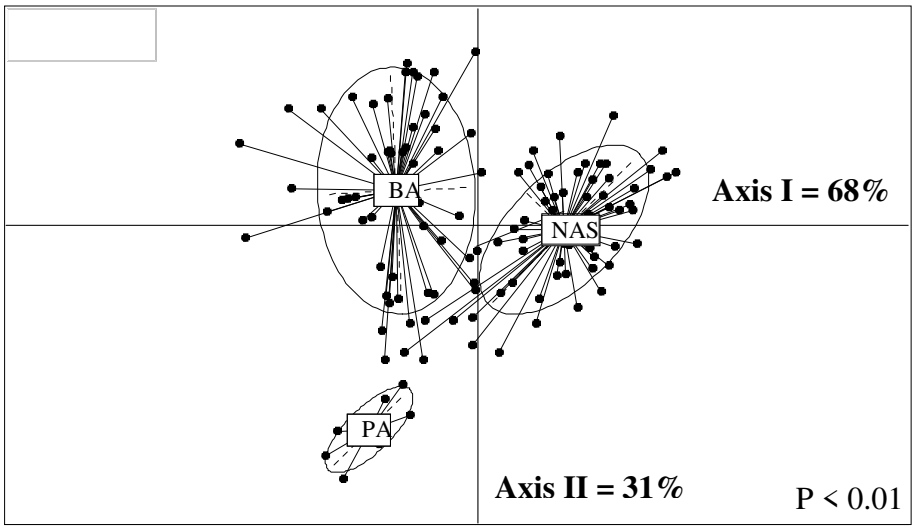

A)
OD
RA
BA
PA
F-2
F-1
9-y
6-y
5-y
1-y
16-y
Axis1 = 31%
St
NSA
Axis 2 = 22
Eigenvalues
40
30
20
10
0
1
2
3
4
5
6
B)
d = 0.5
BA
NSA
St
pH
RA
OD
N
MO
PA
Variables

Figure 1.


BA
NAS
PA
Axis I = 68%
Axis II = 31%
P < 0.01

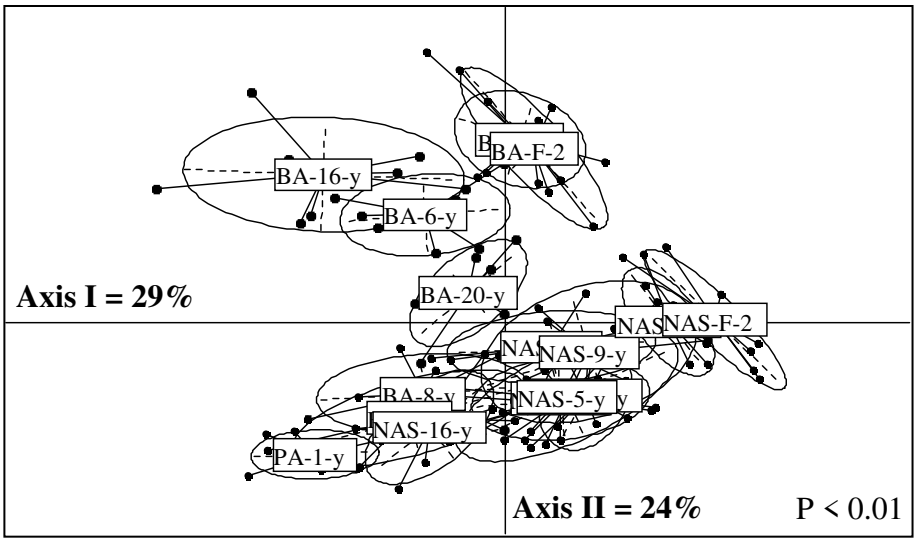

BA-F-2
BA-16-y
BA-6-y
BA-20-y
NAS-F-2
NAS-9-y
NAS-5-y
NAS-16-y
PA-1-y
Axis I = 29%
Axis II = 24%
P < 0.01

Figure 2

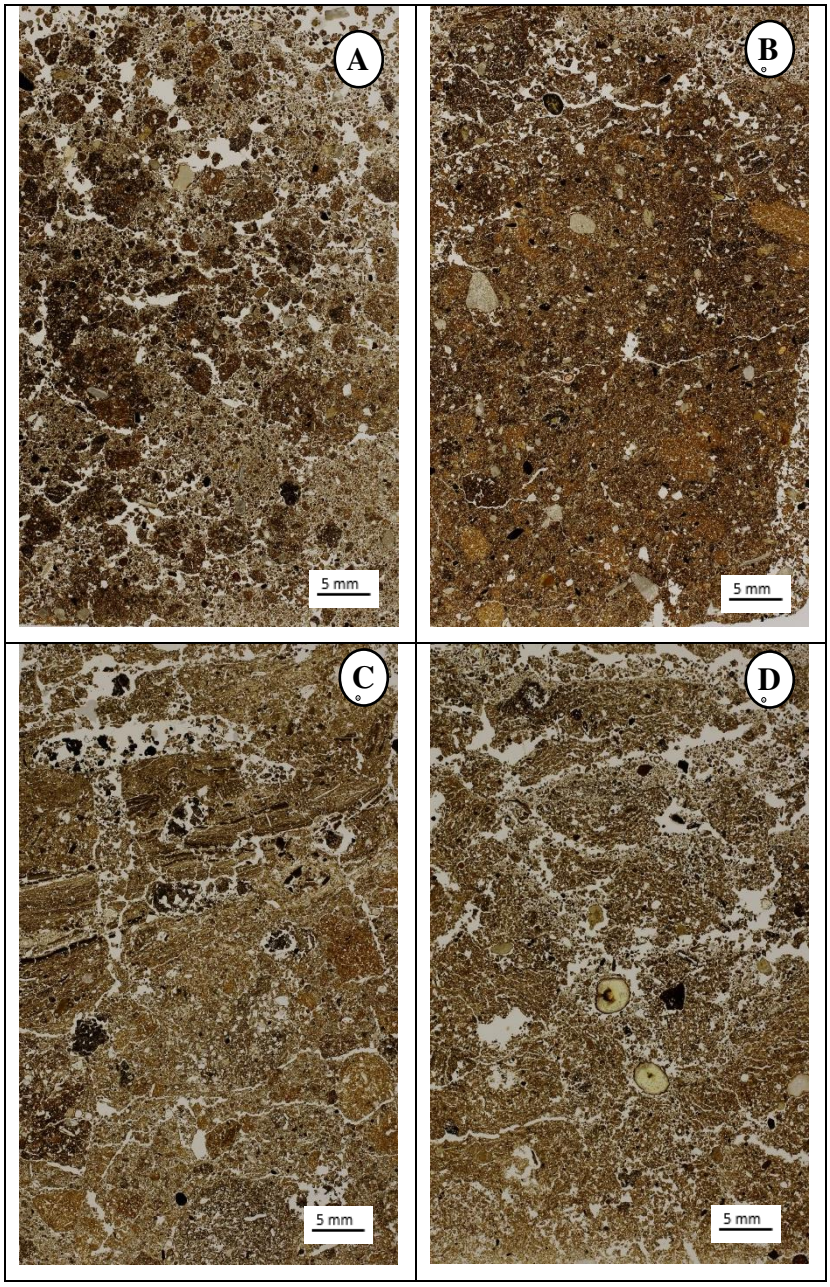


Figure 3.

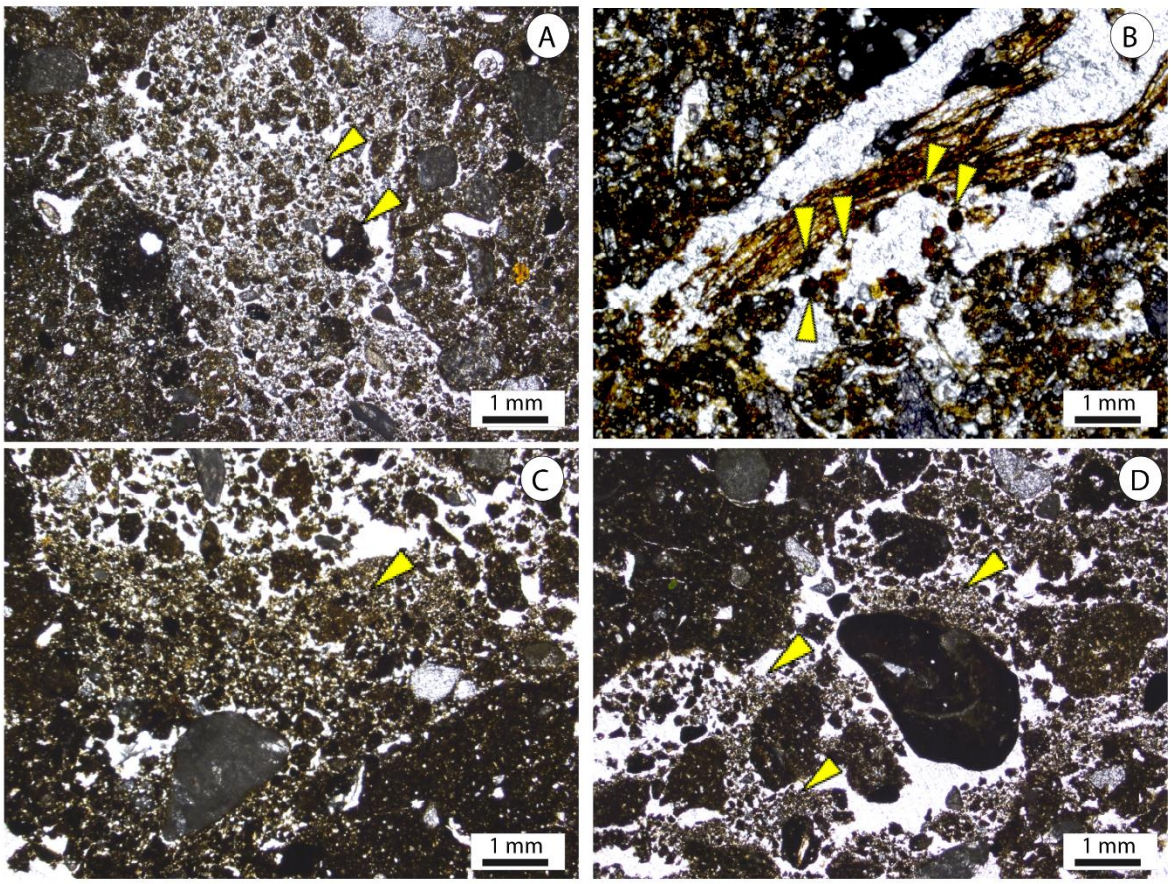


Figure 4

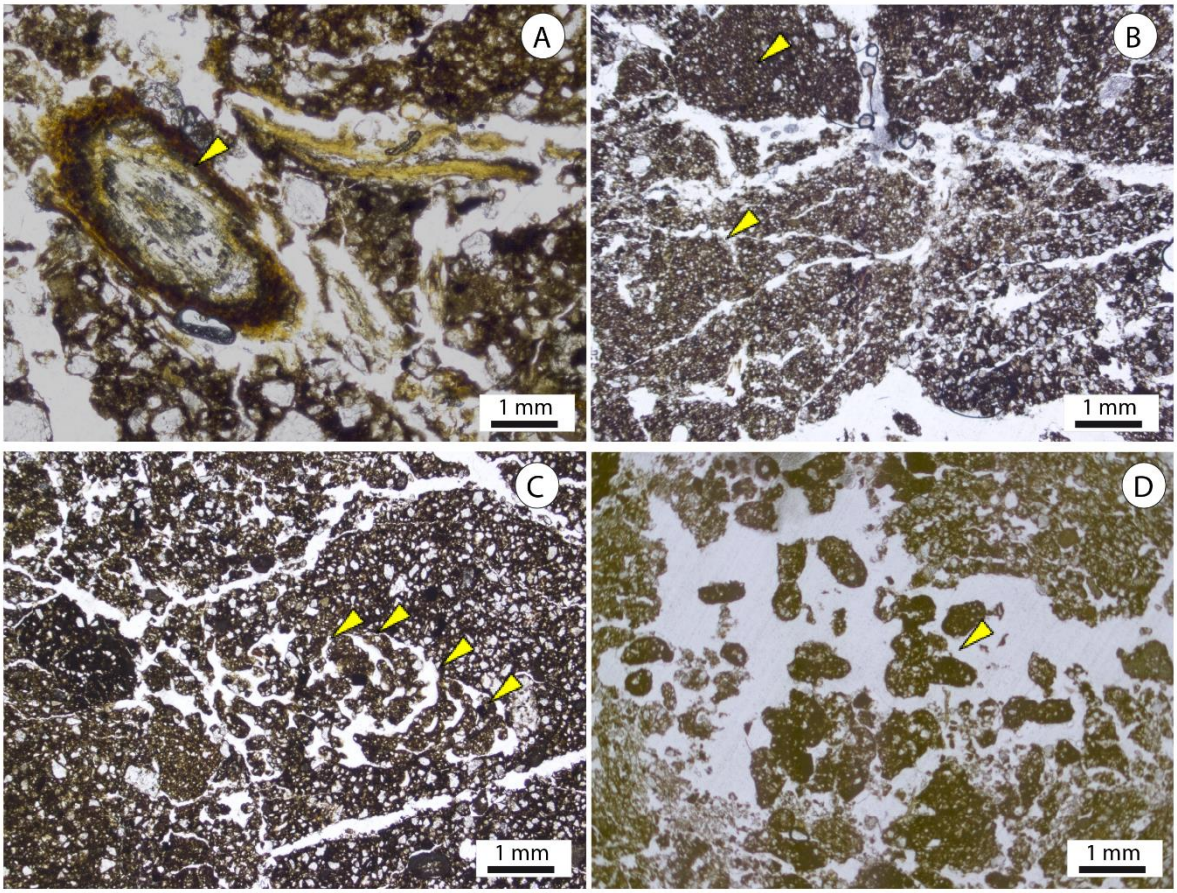


Figure 5

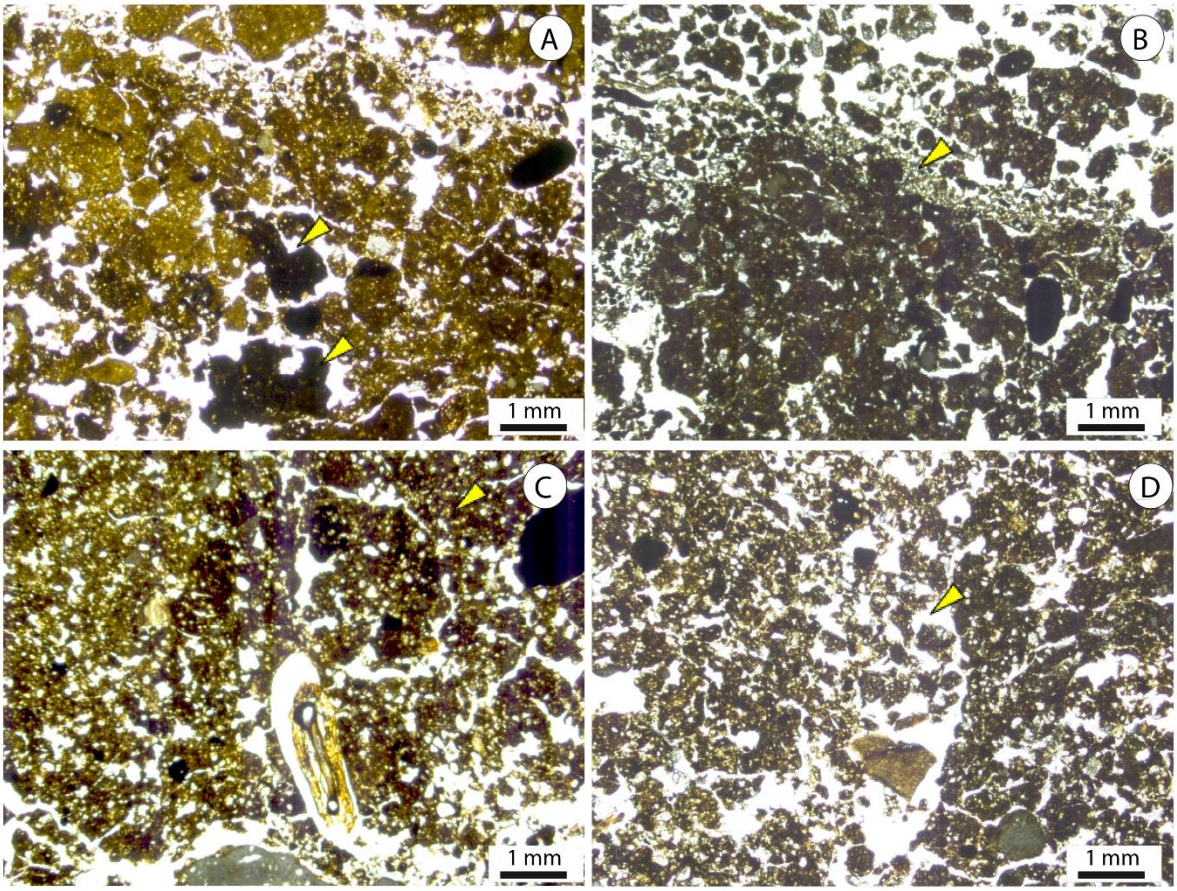


Figure 6

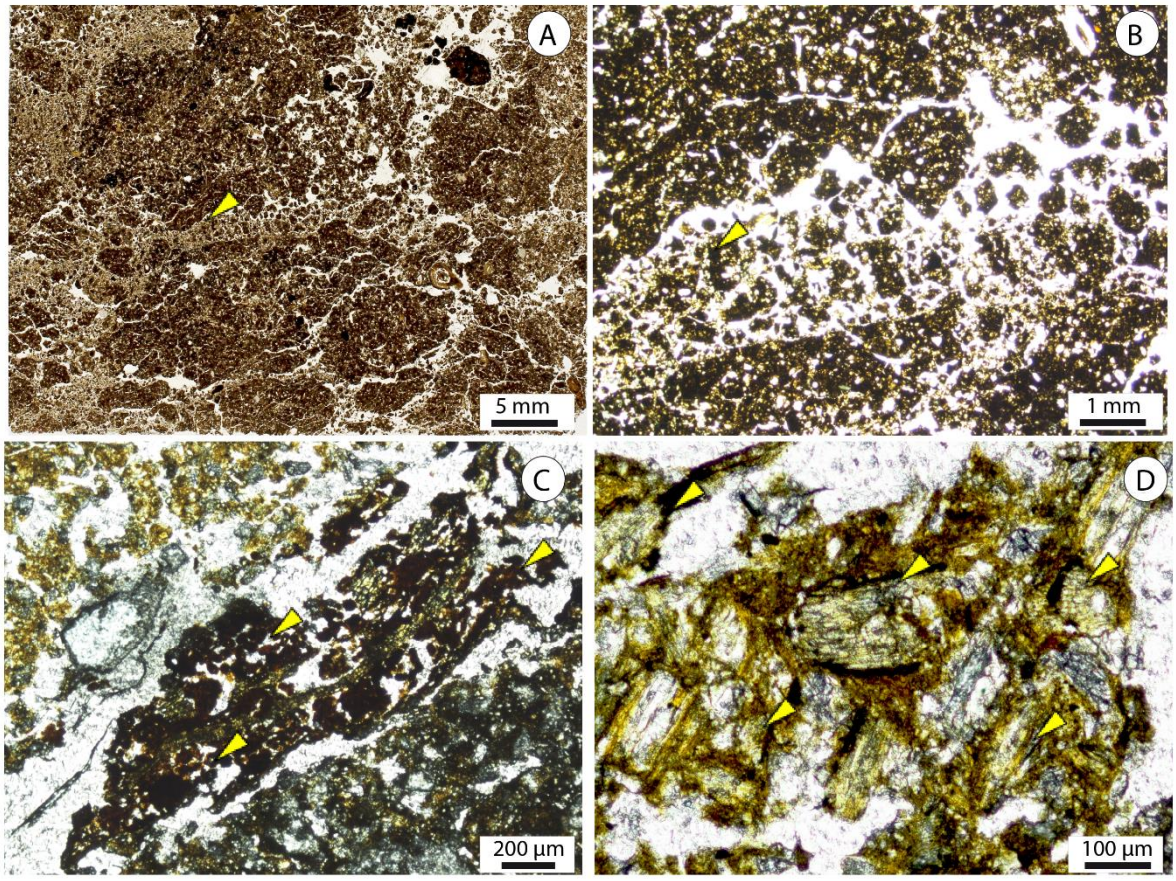
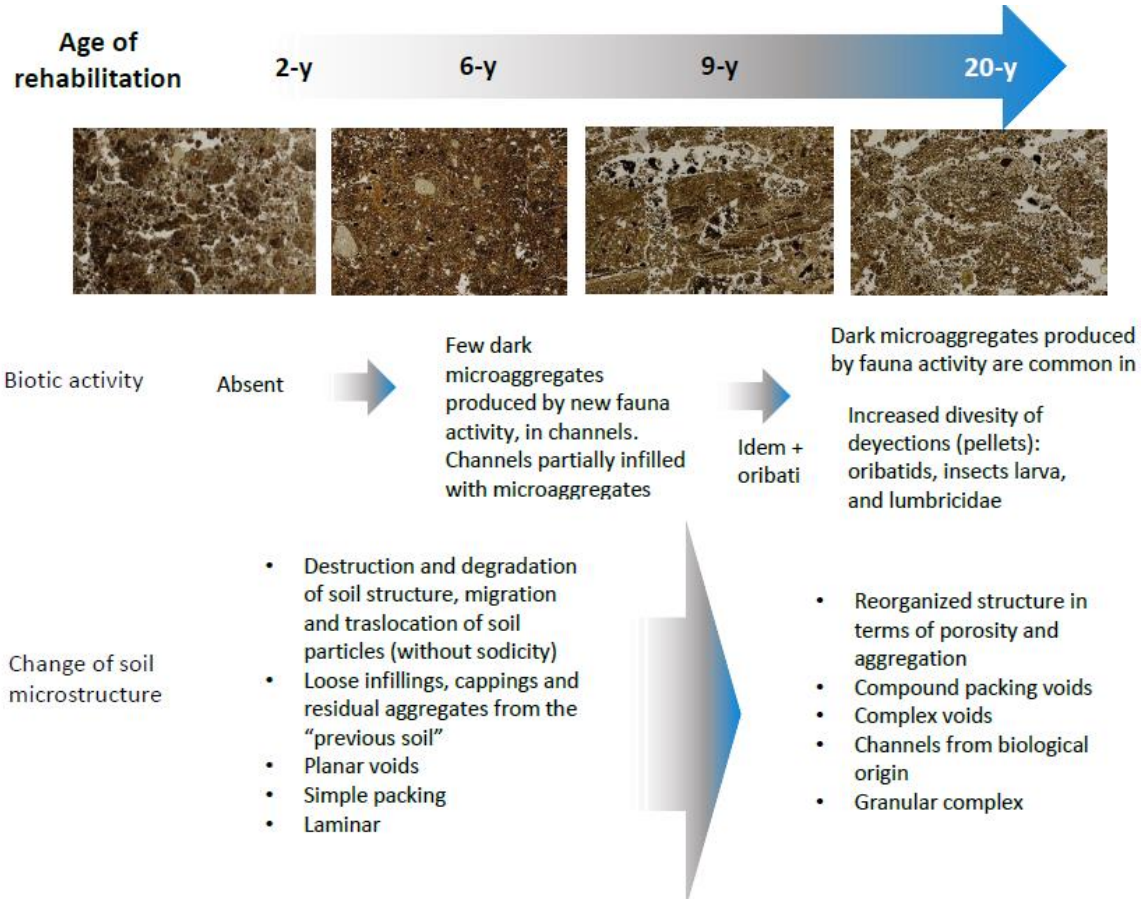


Figure 7



Graphical abstract